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An approach to the determination of N-nitrosodimethylamine at part-per-quadrillion levels using Positive Chemical Ionization and Large-Volume Injection

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Introduction

N-nitrosodimethylamine (NDMA, Figure 1) is one of a series of nitroso compounds known to be carcinogenic. NDMA is found in nitrate-cured or smoked meats,[1] cheeses,[2] tobacco smoke,[3] cooked foods and in beverages such as beer[4] (both foreign and domestic[5–7]). The presence of NDMA in surface waters designated for use drinking water use is of particular concern and the U.S. Environmental Protection Agency (EPA) has promulgated a regulatory standard for these waters of 0.7 ng/l (700 ppq). When in 1998 NDMA was detected in California drinking water, the source was associated with the production and use of a rocket fuel component, unsymmetrical dimethylhydrazine. In response, the California Department of Health Services (DHS) announced an action level in drinking water of 2 ng/l (2 ppt). However, the best available methods in the literature provide *detection* limits on the order of 1–3 ng/l. EPA methods 625 and 1625 specify a detection limit for NDMA of 50 ppb—25,000 times the California DHS action level and 70,000 times the EPA regulatory standard. It follows that using existing methodologies, *any* detection of NDMA represents a violation.

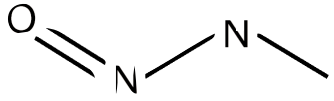

Figure 1. N-nitrosodimethylamine, $(CH_3)_2N_2O$, 74 g/mole, CAS Registry No. 62-75-9

Determining NDMA at ppt or ppq concentrations in water is an analytical challenge. The extraction methods that have been applied, such as liquid-liquid or solid-phase extraction,[8–10] produce concentration factors of 500 to 1000, but overall recoveries are generally low. The high polarity and volatility of NDMA contribute to lowered recoveries and extensive extract concentration by evaporation can lead to high losses.

To increase sensitivity and specificity, one prevalent detection scheme involves use of the chemiluminescent nitrogen detector. Electron impact mass spectrometry has also been used but the fragmentation pattern is not very favorable (Figure 2). While the molecular ion at 74 m/z may be a reliable quantitation ion, the confirming ions at 42 and 43 m/z are hardly unique and are easily compromised by fragments from interferences.

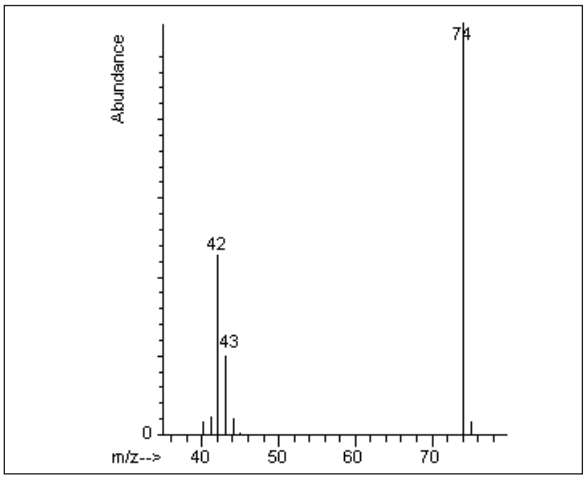

Figure 2. Electron impact ionization mass spectrum of NDMA

One approach to overcoming the unfavorable electron impact (EI) ionization mass spectrum of NDMA is to apply positive chemical ionization (PCI). PCI can provide enhanced analyte selectivity and sensitivity. Utilizing large-volume injection (LVI) should lower the concentration of NDMA that can be detected in an extracted sample. This note describes the combined application of these two techniques as a possible approach to determining NDMA at ppt and ppq concentrations.

Experimental

NDMA standards were made by serial dilution in 1-ml of dichloromethane from a 100 ng/µl standard (Ultra Scientific, Kingstown, RI; part number NS-100). Dichloromethane was chosen as the solvent, because this solvent is used in both liquid-liquid and solid-phase extraction techniques.

Instrumental Section

The 6980 Plus GC / 5973 MSD with chemical ionization option was operated in the selected-ion-monitoring mode (SIM) with ammonia reagent gas. An HP-210 GC column 50%-trifluoropropyl-50%-methyl-siloxane (30-m, .25 mm i.d., 0.5 µm film thickness, Part Number 19091C-733) was used with a 5-m, 0.32 mm i.d. uncoated retention gap (Part Number 19091-60600) joined by a press-fit connector (Part Number 5062-3555). A 100-µl syringe was used in the integrated automated liquid sampler 7683 injector for the 50-µl injections. GC oven conditions and mass selective detector settings are given in Tables 1 and 2.

Table 1. GC and Injector parameters

Oven Temperature Program	Temp	Time
Initial Temperature	45°C	3.00 min
Ramp 50°C / min	180°C	0.50 min
GC Oven Equilibrium Time		3.00 min
MSD Transfer Line		225°C
Inlet Mode		Split
Split Flow		50 ml / min
Gas Saver		Off
Column Flow (Helium carrier gas)		2.0 ml / min
Mode		Constant Flow
Outlet Pressure		Vacuum
Injection Volume		50 µl
Syringe Size		100 µl
Plunger Speed		Slow
Solvent Washes A, B	Methanol*	Dichloromethane

* A solvent that "wets" the glass bore improves syringe life.

Table 2. MSD parameters

Tune File *	PCINH3.U
Ammonia Reagent Gas Flow	10 %
EM Voltage	PCI CH_4 AutoTune + 400V
MS Quadrupole Temp	106°C
MS Source Temp	250°C
Acquisition Mode	SIM
Solvent Delay	5.25 min
SIM Ions	Dwell
75.1 amu	80 msec
92.1 amu	80 msec

* PCI Autotune parameters were used for these experiments. Autotune provides high sensitivity over a large mass range, but even greater sensitivity for these low molecular weight ions can be achieved by manual adjustment of the tuning parameters.

Large-Volume Injections

The APEX ProSep™ 800 Series XT Plus Preseparation System Inlet (APEX Technologies, Cincinnati, OH) was used as the inlet for large-volume injections.[11, 12] Injections were made into a fused-silica preseparation column packed with deactivated fused-silica wool in the top 3 to 7 cm of the column (available from APEX). The ProSep Precolumn Temperature Module and Flow Module parameters that were successful for this particular preseparation column are given in Tables 3 and 4. This is a very flexible device, and the parameters given can be further optimized to provide better performance for particular extracted matrices. For example, a higher final precolumn temperature than 180°C can be applied to remove high-boiling contaminants.

Table 3. ProSep Precolumn Temperature Program

	Target	Duration
Initial	45°C	0.05 min
250°C / min	180°C #	6.00 min

Higher bake-out temperatures are recommended for extracted samples.

Table 4. ProSep Precolumn Mode Program

	Mode	Duration
Initial	Split	0.05 min
1	Splitless	0.07 min*
2	GC Split **	2.50 min*

*These times should be appropriately optimized.

** It is recommended that ProSep Split be implemented instead of simply GC Split due to superior venting.

Results

The application of PCI with ammonia reagent gas to NMDA produces a simplified mass spectrum consisting only of protonated NDMA, $[NDMA+H]^+$, and the ammonium adduct, $[NDMA+NH_4]^+$, which correspond to 75 m/z and 92 m/z, respectively. PCI provides a three-fold advantage over the EI approach. First, the relatively non-unique 74, 43, 42 m/z ions of the EI have been replaced by higher-mass ions. Second, PCI provides increased sensitivity for NDMA and a reduction in low-mass, "background" ions which enhances the signal-to-noise ratio. Third, by manipulating the ammonia gas flow, the abundances of the 92 m/z and 75 m/z ions can be controlled. As the ammonia flow into the source is increased, the abundance of the $[NDMA+NH_4]^+$ adduct also increases, allowing the ratio of 92 m/z to 75 m/z to be controlled by the analyst. For example, at 0.4 ml/min of ammonia—a relatively low flow setting of the reagent gas mass flow controller (8% of the total 5-ml/min provided by the controller)—the ratio of the protonated form to adduct is biased toward the protonated form: $[NDMA+H]^+$ **:** $[NDMA+NH_4]^+ = 4$ **:** 3. At higher flows, the situation reverses and $[NDMA+NH_4]^+$ predominates, e.g., at 0.9 ml/min ammonia (18% flow setting) $[NDMA+H]^+$ **:** $[NDMA+NH_4]^+ = 1$ **:** 5. It is therefore possible to produce an intense confirming ion for quantitative applications. A good compromise between signal intensities and ion abundancies was achieved at a 0.5 ml/min ammonia flow setting. Figure 3 shows the 75 m/z and 92 m/z SIM signals for a 40-fg/µL standard for this flow. Under these conditions, $[NDMA+H]^+$ is 79% of $[NDMA+NH_4]^+$ according to the integrated signal areas.

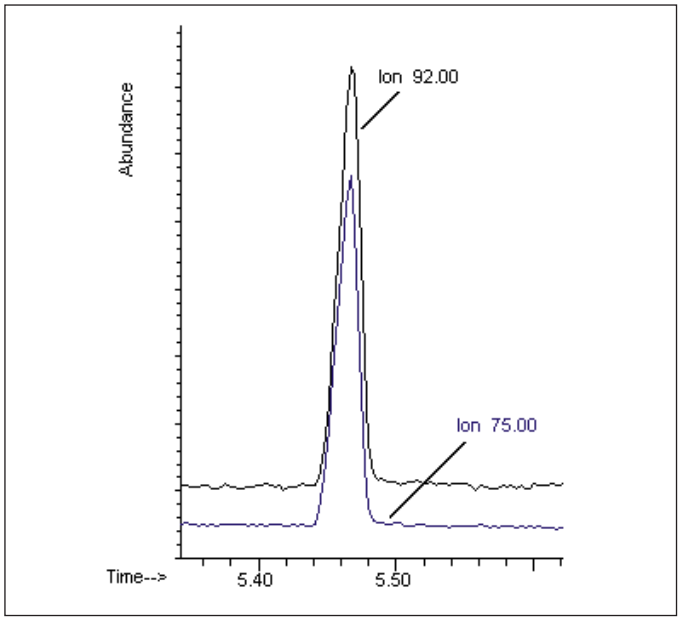

Figure 3. Extracted ion chromatogram for NDMA at 40-fg/µl using PCI-SIM with NH_3 reagent gas.

Figure 4 shows the results of a linear regression of the response of the 92 m/z ion for 50-µl injections of NDMA standards from 20-fg/µl to 4000-fg/µl. The regression fit was very good, $r^2 = 0.999$, considering the propagation of error in the dilutions. The relative standard deviation in the response factors was less than 6% and could be improved by using a perdeuterated or ^{15}N-labeled NDMA surrogate.

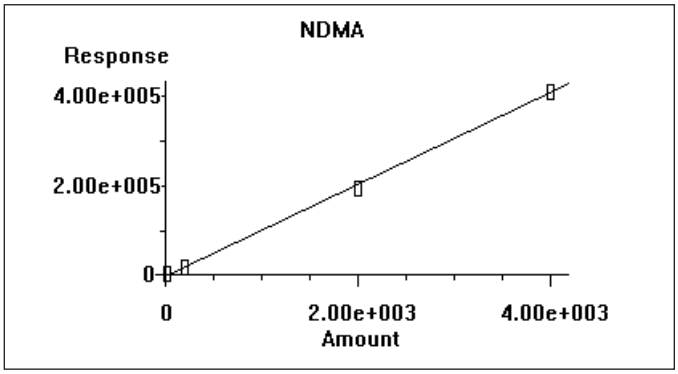

Figure 4. Linear regression of response of the 92 m/z ion versus NDMA concentration from 20-fg/µl to 4000 fg/µl, $r^2 = 0.999$.

Table 5 shows the excellent degree of reproducibility in the ratio of 75 m/z confirming ion to 92 m/z target ion over a wide range of concentrations. The absolute value of the ratio was 0.79, with a relative standard deviation of < 3%. This high precision is important to the degree of confidence in confirming and quantitating NDMA.

Table 5. Reproducibilities of the ratio of the integrated areas of 75 m/z : 92 m/z and the response of the 92 m/z target ion for 5 injections at 5 concentrations.

Concentration as fg NDMA / µl	RSD Ratio 75 mz / 92 m/z	RSD Response by 92 m/z area
20	2.9%	2.4%
40	2.2%	3.2%
200	0.7%	0.8%
2000	0.7%	1.7%
4000	0.3%	0.9%

Table 5 also shows the excellent reproducibility of the response of the 92 m/z ion for replicate 50-µl injections. Even at the 20-fg/µl concentration, precision is better than 3%.

Conclusions

Concentration and recovery factors for NDMA using present published methodologies suggest effective preconcentration of NDMA in samples to be on the order of 500, e.g., 60–70% recovery of NDMA in extraction of a 1-liter water sample. This implies that the low 20-fg/µl NDMA standard corresponds to a sample concentration of 40 pg/l, or 40 parts-per-quadrillion. Alternatively, to quantitate NDMA at 0.5 ppt in water, which is 4 times lower than the California DHS limit and slightly lower than the EPA regulated limit, quantitating at 20 fg/µl is equivalent to requiring the extraction of *only* 80 ml of water even if recoveries are still only 50%. Extracting small volumes presents a significant simplification of the process and offers savings in solvent and related materials, and in processing time.

With NDMA eluting in about 5 1/2 minutes, the analysis is fast, and the run-to-run cycle time is short—less than 13 minutes between injections. The method may be further optimized for even more rapid analysis.

The 5973 MSD provides very stable ratios for the confirming ion that can be optimized for quantitative purposes as described. In contrast to EI, in which many possible interfering fragment ions are possible that may distort the ratio of the target and confirming ion(s), PCI with ammonia is unlikely to cause fragmentation-induced interferences because of the relatively "gentle" nature of ammonia reagent gas. Interferences could occur involving compounds with molecular weights of 74 or 91 g/mole eluting at the same retention time but that is unlikely scenario.

The high degree of reproducibility in the injections, even at very low NDMA concentrations, demonstrates the robustness of large-volume injections using the APEX ProSep with the 6890/5973 MSD. It should be emphasized that the reproducibility of 2.4% for the replicate 50-µl injections of the 20-fg/µl standard reported here was for the *absolute* response. Use of an internal standard should further lower the deviation in response and improve quantitation.

Using this approach it should now be possible to satisfy the 2 ppt action level for NDMA set by the State of California and the 700 ppq regulatory standard promulgated by the U.S. EPA.

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